UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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12-MAR-1986

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Glyphosate; EPA Registration No. 524-308; Roundup; Additional Histopathological Evaluations of Kidneys in the Chronic Feeding Study of Glyphosate in Mice.

Caswell No. 661A
Accession No. 260023

TO: Robert J. Taylor
Product Manager (25)
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Edwin Budd
Head, Review Section II
Toxicology Branch
Hazard Evaluation Division, (TS-769C)

FROM: William Dykstra
Toxicology Branch
Hazard Evaluation Division, (TS-769C)

William Dykstra 1/7/86

Requested Action:

Review additional pathological and statistical information on kidney tumors with glyphosate.

Background:

Glyphosate was considered oncogenic in male mice causing renal tubule adenomas, a rare tumor, in a dose-related manner. The incidence of this tumor was 0, 0, 1, and 3 in the control, low-, mid-, and high-dose groups, respectively.

Additional evaluation of all original renal sections by Dr. Kuschner identified a small renal tubule adenoma in one control male (animal no. 1028) which was not diagnosed as such in the original pathology report.

Subsequently, Toxicology Branch recommended that additional renal sections be cut and evaluated from all control and glyphosate treated male mice in order to determine if additional tumors were present.

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The results of the additional pathological evaluation of re-cut kidney sections in male mice demonstrated that no additional tumors were present. Additionally, the tumor in the control group (animal number 1028) which had been diagnosed from the reevaluation of the original slides by Dr. Kuschner was not present in the re-cut kidney sections. Therefore, the following incidence was observed.

Dose (ppm)	0	1000	5000	30,000
Renal tumors	0, 1*	0	1	3
No. examined	49	49	50	50

*Animal (number 1028) which was diagnosed by Dr. Kuschner as a renal tumor after reevaluation of original slides but not of resectioned kidney slides.

Conclusions:

The additional pathological and statistical evaluations by consultants conclude that the renal tumors in male mice were not compound-related.

This information will be submitted to the TB Ad Hoc committee for evaluation to determine if concurrence is possible.

Review:

1. Letter of September 29 1985, Robert A. Squire, D.V.M., Ph.D., to Monsanto.

Dr. Squire has not evaluated the slides of the glyphosate study but rather the chronic toxicity data.

The following is the narrative from Dr. Squire's letter:

"The pathological endpoint in question is the presence of renal tubular adenomas in male mice. The final overall incidences were 1/49, 0/49, 1/50, and 3/50 for control, low, mid, and high doses respectively. In my opinion, these represent spontaneous occurrences rather than compound-related effects. This view is based primarily upon the biological and pathological evidence available, but is also supported by the lack of statistical significance, either in comparing proportions of animals affected or linear trend analyses."

"The following observations suggest to me that the findings in male mouse kidneys are incidental to treatment:

"A. Historical control values in the three different laboratories indicate that, although renal tubular neoplasms are relatively rare in mice, they do occur sporadically and there is considerable variation from group to group. An analysis of these tumors should combine the adenomas and carcinomas since they represent a spectrum in development and the lesion classification is uncertain. If one does this with the Hazelton Laboratory data, there is an overall incidence of 5.4 percent tubular neoplasms which is essentially the same as the high dose animals in your study. The incidence of tubular carcinomas is not listed for Biodynamics laboratory and IRDC shows very low incidences. However, it must be kept in mind that the historical control data are derived from studies in which there were the customary one or perhaps two kidneys sections examined. If four sections had been taken from each kidney, as in your study, it is likely that historical control incidences would have been even higher."

"B. Based upon Dr. Kuschner's histopathological evaluation of the kidney slides, no preneoplastic or cytotoxic changes were evident. I know of no instance where a renal carcinogen was given at a dose sufficient to induce tumors without also inducing tubular toxicity and hyperplasia, not only in the tumor-bearing animals, but in many additional animals receiving the same exposure levels. Carcinogenesis is multi-stage process beginning with hyperplasia, and when a population of animals is exposed to a tumorigenic dose, many develop early stages of neoplastic progression even though only a few may reach the final stage, i.e., tumors. The absence of preneoplastic changes virtually precludes this being a compound-related effect."

"C. The largest and most atypical tumor in the study, according to Dr. Kuschner, was an animal in the mid-dose group (#3023). This would be highly unlikely if the tumors were compound-related since one expects the most advanced tumors to be in animals receiving the highest dose of carcinogen. Carcinogens increase not only the incidence, but the degree of neoplastic progression. This is particularly true here since survival in the high dose males exceeded that of control animals."

"In summary, I feel the weight of evidence strongly suggests that the renal adenomas in male mice were naturally-occurring and not treatment related."

2. Letter of October 3, 1985, from Marvin Kuschner to Monsanto.

In this letter, Dr. Kuschner states that he has asked Dr. Andre Varma, Chairman of the Department of Community and Preventive Medicine and a well-known biometrician, to examine the data.

The narrative of Dr. Varma's letter of October 10, 1985 to Dr. Kuschner is presented below:

"Statistical Analysis

"A chi-square analysis of these data is not valid, because the necessary assumption of an approximate normal distribution is not valid with these small numbers. The exact Fisher's tests to compare the mice fed glyphosate with the control group is valid, but does not allow one to study the possible dose-response relation. A probit-type analysis is not appropriate with the low responses. Furthermore, there is a baseline no-dose response of one tumor in forty-nine (49) mice."

"I have decided to use a randomization test to study the dose-response. The experiment is treated as an occupancy problem. A total of five (5) tumors were observed among the male mice. I will assume that the chance of the four groups of mice is proportional to the number of mice in the group under the null-hypothesis of no effect of the glyphosate. Thus the chance of a tumor in the control group and in the 1000 ppm group is 1/49 and 1/50 in the 5000 and 30,000 ppm groups."

"Table 1 list all the 56 possible distributions of the five tumors in the four groups of mice and the associated probabilities. The chance of observing the "1 0 1 3" configuration of tumors is 0.020127. The chance of observing configurations as rare as this one or with smaller probabilities, i.e., all configurations with $p \leq 0.020127$ is 0.414134. The "1 0 1 3" configuration is therefore, not a rare event."

"I am using the following criteria to conclude that a configuration corresponds to a dose-response."

1) No response in the control group.
2) No higher response rate at a low dose.
3) No lower response rate at a higher dose.

"Using these criteria the following configurations are considered to indicate an increasing dose-response to the preparation:"

0 0 1 4
0 0 2 3
0 1 2 2
0 1 1 3

"The sum of the corresponding probabilities of these four configurations is 0.065720. The 1 0 1 3 configuration is not considered to indicate a dose-response according to the criteria listed above. If its probability is added to the set, the total chance of dose-response permutations becomes 0.085847."

"Based on the analyses outlined above there is no evidence of a statistically significant trend in the proportion of mice with renal tumors as a response to the dose of glyphosate in their diet."

3. Letter of October 7, 1985, from Robert E. Olson, M.D., Ph.D. to Monsanto.

The narrative of the letter is presented below:

"In response to your letter of September 16th asking me to evaluate the glyphosate mouse kidney adenoma study, I am pleased to respond. Let me begin by saying that the evidence for carcinogenicity of glyphosate in mice is unconvincing. A few of renal adenomas were found in male but not female mice given very large doses of the compound, i.e., 5,000 and 30,000 ppm in the diet, corresponding to 0.5 and 3.0 percent of the diet. The distribution of tumors in the three groups of male mice studies were 1/49 in the control group 0/49 in the group fed 1,000 ppm, 1/0 in the group fed 5,000 ppm, and 3/50 in the group fed 30,000 ppm. There were no tumors in any of the female mice. These data suggest that the appearance of these tumors is random and not dose-related."

"I am further impressed by the fact that a restudy of kidneys from mice in the study by Dr. Kuschner, a world-famous pathologist, has confirmed the original findings and found no new tumors, despite the fact that three additional sections per kidney, per mouse, spaced at 150 microns intervals were evaluated. This indicates that the density of tumors in both experimental and control groups is very low and supports the view that these are spontaneously developing tumors at a very low frequency."

"When one examines other control groups, one finds that the renal adenoma is not a rare tumor in untreated mice of the same CD-1 strain and that in seven studies by Biodynamics over the past several years, renal adenomas have been observed in the control groups in two of these studies--Study A (1/54 or 1.9 percent) and E (2/60 or 3.3 percent). The control group incidence in comparable studies by International Research and Development was 0 to 1.4 percent, and at Hazeltine, the control mice exhibited this tumor at rates of 7.1 percent (1/14)."

"In summary, it is my view that these findings do not support the view that glyphosate is oncogenic in mice. These results would not be accepted by any peer-review journal as evidence of carcinogenicity. To me, it represents a negative result, which would not be regarded by any scientific group or reputable agency as evidence of carcinogenicity."

4. Letter of October 17, 1985, from Klaus L. Stemmer, M.D. to Monsanto.

"In your letter of September 17, 1985, you requested an evaluation of the material, submitted with the letter, of the mouse kidney tumor data found in the chronic feeding study of glyphosate. In addition, I received the kidney sections of the male and female mice of this experiment."

"I reviewed the kidney slides of the male mice and confirmed the findings of renal tubular neoplasms in the following five (5) animals: 1028, 3023, 4029, 4032, and 4041. These tumors were cytologically well differentiated. I could not verify any pre-malignant features in the renal tubular epithelium of any of the experimental mice. Intercurrent renal diseases, which were noticed, did not support any cytotoxic effect of the test material. Also, no histologic changes were present suggesting that the test material might enhance carcinogenesis."

"The final report furnished by Bio/dynamics Inc. on July 21, 1983, does not enumerate any pathologic alterations in the kidneys of the male mice that could be interpreted as enhancement of the development of neoplasms (pages P1 to P17 of report). I am certain that the pathologists examined the kidneys for lesions of that nature since they did and reported them for the liver. The lack of finding such changes supports the statement in the previous paragraph and in the report of M. Kuschner, M.D."

"The historical data on the incidence of renal tubular adenomas were reviewed. Bio/dynamics Inc. reported a percentage range from 0 to 3.3 percent; International Research and Development Corporation found a percentage range from 1 to 2 percent, and Hazelton had a range from 0 to 3.6 percent. In the present chronic feeding study, the incidence in control male mice was 2 percent. As is stated in the Hazelton report, the expected percentage incidence could be as high as 7 percent. On the basis of these data, the occurrence of three renal tubular adenomas in the high dose group (6%) would still fall into the general percentage range of male control CD-1 mice."

"The data in appendices 17 A and 17 B strongly indicate that the CD-1 mouse has a high incidental occurrence of neoplasms in many different organs (report of Bio/dynamics Inc.). The incidence is: control 20 out of 50 mice, low dose 28 out of 50, medium dose 29 out of 50, and high dose 24 out of 50. In evaluating the potential tumorgenicity or carcinogenicity of the test compound one should take this into consideration. It might be that one can find a slight statistical significance in the "dose related" data, if one ignores the historical data (previous paragraph). Whether this has any biological significance is doubtful. In the CD-1 mouse having a high occurrence of neoplasms, the "dose related" incidence of renal tubular adenomas is in all probability biologically by chance."

5. Letter of October 10, 1985, from Pathology Working Group (PWG) to Monsanto.

Participants in the PWG

Dr. R. M. Sauer (Chairperson)
Dr. M. R. Anver
Dr. J. D. Strandberg
Dr. J. M. Ward
Dr. D. G. Goodman

Conduct of the PWG Review

"Prior to the PWG review, the Chairperson reviewed the pathology incidence tables, the original pathologist's (OP) narrative, pertinent individual animal records and all tissue sections of kidneys from male mice. The letter included the original set of kidney sections which were read both by the OP and Dr. Kuschner and a subsequently prepared set of 3 step sections from each kidney block which had been read by the OP. The kidney was the designated target organ for the PWG review."

"The PWG blindly examined coded slides without respect to treatment group of all cases or renal tubular-cell tumors and all discrepancies in diagnoses among the OP, Dr. Kuschner and the Chairperson of renal tubular-cell tumors and renal tubular-cell hyperplasias. The consensus viewpoint of the participants is recorded in Appendix A."

"The PWG also reviewed all sections of kidneys from control and high dose males for incidence and severity of naturally occurring conditions and induced toxic lesions."

Comments and Recommendation of the PWG

"Microscopically, tubular cell adenomas are well circumscribed and compress the adjacent parenchyma. They are composed of variably sized cuboidal, columnar or polygonal cells which form solid lobules separated by delicate connective tissue septa. The cytoplasm may be basophilic but is usually eosinophilic and granular or vaculated and reticular. The nuclei are round and open faced. Mitoses are infrequent."

"Tubular-cell carcinomas are usually larger and may invade the adjacent parenchyma. The cells are more pleomorphic than in the adenomas and often contain large bizarre nuclei. Mitoses, while not common, are more frequent than in adenomas. Necrosis, hemorrhage and cholesterol clefts are often present."

"Renal tubular-cell hyperplasia consists of a small circumscribed lesion with or without increased basophilia and an increased number of nuclei piling up and filling the lumen. There is usually some expansion of the tubule and loss of tubular architecture but without compression of adjacent parenchyma. Typically the cells have poorly defined cytoplasmic borders, round open-faced nuclei and have a relatively high nuclear/cytoplasmic ratio."

"The incidence of renal tubular-cell neoplasms as determined by the PWG is presented in Table I. Because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type it is appropriate to combine the incidences for purposes of evaluation and statistical analysis."

TABLE I
RENAL TUBULAR-CELL LESIONS

	Male Mice			
	Control	Low Dose	Medium Dose	High Dose
Tubular-cell adenoma	1	0	0	1
Tubular-cell carcinoma	0	0	1	2
Combined incidence	1	0	1	3

"This PWG firmly believes and unanimously concurs with the original pathologist and reviewing pathologist that the incidences of renal tubular-cell neoplasms in this study are not compound related."

"The following points were taken into consideration in reaching this decision:"

"a) Renal tubular-cell tumors are spontaneous lesions for which there is a paucity of historical control data for this mouse stock. However, clustering can occur and the incidence in this study is comparable to the available historical control range from several laboratories (Appendix B). Since there were 3 treated groups and only 1 control group, there is a greater possibility of more variation from mean control incidences in the treated mice."

"b) None of the treatment groups differed from the controls by the Fisher exact test at the 0.05 level of significance. Over all groups there was no evidence of a significant linear trend at the 0.05 level by a one-tailed Cochran-Armitage Test."

"c) Multiple renal tumors were not found in any animal."

"d) Compound related nephrotoxic lesions, including preneoplastic changes, were not present in this study. In addition, renal toxicity was not noted in the 3-month subchronic toxicity study reported in December 1979."

"Spontaneous chronic renal disease is commonly seen in aged mice. It consists of a spectrum of lesions which may occur individually or in various combinations in any particular kidney. Individual lesions reported by the OP in this study and listed in his updated report may be components of this complex. Chronic interstitial nephritis, a term used by the OP, is a summary and redundant diagnosis which encompasses several of the individual components and should not be singled out for statistical analysis."

"Many animals in this study had proliferative, cystic lesions of the parietal layer of Bowman's capsule and of the proximal convoluted tubules. These changes were apparently more severe in control than treated animals."

"Based on the review of all high dose and control male kidneys, the PWG did not observe an increase in incidence or severity of non-neoplastic lesions in the kidney of high dose animals. The PWG concurs with the OP that there is no evidence that these lesions were compound induced or related."

END